time then is about 5 million years, if equal duration of early, middle, and late Miocene be assumed. Should a species, which had a brief larval planktonic stage, have arisen and become extinct during Miocene time, its geographic range may be restricted. The necessary dating ordinarily cannot be fixed within narrow enough limits to evaluate this factor.

There is bound to be a hard core of unqualified endemism, just as in modern faunas.

Owing chiefly to insufficient sampling and the vagaries of burial and preservation, much of the endemism is apparent rather than real. Many examples of widely discontinuous distribution of species and genera, which are not relicts, support that conclusion. *Murex textilis*, the sole species of the subgenus *Subterynotus*, is found in the middle Miocene of the Dominican Republic and Venezuela, and then appears in the Pliocene of Florida, although there are no Miocene records in Florida. Two unrelated species of *Gemmula (G. vaningeni* and *G. machapoorensis)* occur in Trinidad, the Canal Zone, and Florida, but nowhere between those three areas. The occurrence of a mangrove ark (*Anadara tuberculosa*), now living in eastern Pacific waters, in deposits of late Pliocene age in Florida (*4*) is a striking example of missing records. The only rational explanation for that occurrence is that this species migrated through the western Atlantic part of the Miocene Caribbean province. Yet neither the species nor its predecessor has so far been found there.

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References and Notes

1. W. M. Gabb, *Trans. Am. Phil. Soc.* **15**, 200–257 (1873).
2. A. A. Olsson, *Bull. Am. Paleontol.* **9**, 25 (table), 27 (1922).
3. W. P. Woodring, *Carnegie Inst. Wash. Publ.* **366**, 108 (1928).
4. D. Wilson, in A. A. Olsson, *Bull. Am. Paleontol.* **47**, 521 (1964).
5. C. J. Maury, *Bull. Am. Paleontol.* **5**, 161–478 (1917).
6. H. A. Pilsbry, *Proc. Acad. Nat. Sci. Phila.* **73**, 305–435 (1922).
7. W. P. Woodring, *Carnegie Inst. Wash. Publ.* **366**, 1–222 (1925); *ibid.* **385**, 1–564 (1928).
8. C. J. Maury, *Bull. Am. Paleontol.* **10**, 153–410 (1925).
9. R. Rutsch, private report, pp. 1–75 (1940).
10. P. Jung, *Paleontol. Res. Inst.*, in press.
11. F. M. Anderson, *Proc. Calif. Acad. Sci.* **18**, 73–213 (1929).
12. E. Böse, *Bol. Inst. Geol. Mex.* **22**, 1–96 (1906).
13. M. C. Perilliat Montoya, *Paleontol. Mex.* **8**, 1–38 (1960); *ibid.* **14**, 1–45 (1963).
14. A. A. Olsson, *Bull. Am. Paleontol.* **9**, 1–311 (1922).
15. W. P. Woodring, *U.S. Geol. Survey Profess. Paper* **306**, 1–145 (1957); *ibid.*, pp. 146–239 (1959); *ibid.*, pp. 240–297 (1964).
16. T. Oinomikado, *J. Geol. Soc. Japan* **46**, 103–116 (1939).
17. J. G. Marks, *Bull. Am. Paleontol.* **33**, 1–162 (1951).
18. A. A. Olsson, *Neogene Mollusks From Northwestern Ecuador* (Paleontol. Res. Inst., Ithaca, New York, 1964), pp. 1–256.
19. ———, *Bull. Am. Paleontol.* **19**, 1–272 (1932).
20. The nonendemic and endemic species were tabulated by Barbara A. Bedette, and the illustration was drafted by Elinor Stromberg.

15 December 1964

Lungfish Burrows from the Michigan Coal Basin

Abstract. *Five casts of lungfish burrows have been found in a quarry near Grand Ledge, Michigan, in shale of the Saginaw group, Middle Pennsylvanian. The burrows contain no fish remains, but they closely resemble lungfish burrows from the Lower Permian of Texas which contain remains of the genus* Gnathorhiza.

Of the three living genera of lungfish, two (*Lepidosiren* from South America and *Protopterus* from Africa) are known to aestivate. Both genera burrow into the mud beneath ponds, where they may remain buried without desiccation for over a year. Evidence has been obtained of the occurrence of aestivation among lungfish, from deposits as early as the Lower Permian. Romer and Olson (*1*) described numerous casts of lungfish burrows from the Clear Fork group of the Texas Permian, some containing lungfish of the genus *Gnathorhiza*; however, most of the burrows were empty. Other empty casts of lungfish burrows were recorded from the Lower Permian of Prince Edward Island by Langston (*2*), and from New Mexico by Vaughn (*3*). Judging from the size and shape of the burrows, all may have been made by members of the same genus. The burrows are all of about the same dimensions, 4.5 to 10 cm in diameter and up to 45 cm in length. They are essentially straight, with the base somewhat narrower than the remainder. Unlike living lungfish, which coil themselves up (*4*), *Gnathorhiza* remained straightened out, with its tail at the base of the burrow.

In 1963, while collecting plant fossils from one of the quarries of the Grand Ledge Clay Products Company near Grand Ledge, Michigan, I found five large, erect cylinders embedded in a soft shale. The most complete specimen is shown in Fig. 1. All are similar in appearance to the Permian lungfish burrows, but have a somewhat greater diameter. The burrows are approximately 15 cm in diameter at the upper end. The longest remaining portion is 41 cm in length, but the uppermost portions of all the burrows were damaged during excavation of the quarry. The bases of three are intact, showing a roughly spiral pattern on the surface, presumably made by the fish's tail when digging the burrow. Areas of various portions of the burrows are marked by slickensides, also noted in the Texas burrows by Romer and Olson.

The lower block of each burrow was broken open in the laboratory. No bone was found, but there were fragments of plant material, together with small amounts of iron pyrite and crystalline calcite. The burrows are considerably harder than the surrounding shale and have a higher sand content. As in the casts of burrows described by Vaughn, the central portions of the Michigan casts are a lighter color than the outside, apparently because of differential oxidation of the sediments.

The quarry in which the lungfish burrows were found is situated just west of the Grand River in section 34, Eagle Township, Clinton County,

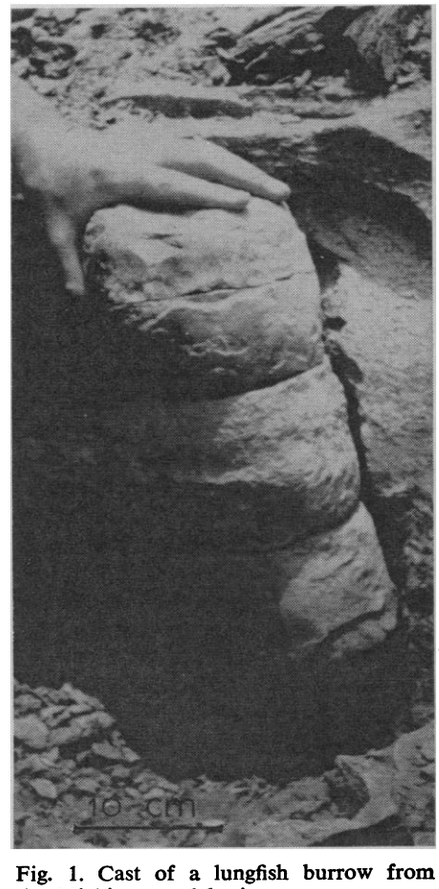

Fig. 1. Cast of a lungfish burrow from the Michigan coal basin.

Michigan. The burrows were at the margin of a pit dug in the base of the quarry. All lay within about 20 meters of each other; two were somewhat displaced by digging equipment, but all had come from approximately the same level. The character of the matrix appears to be uniform from the base of the pit to a few meters above the burrows. It is a soft, light gray shale used in making vitrified tiles. Since the burrows were discovered, this pit has become filled with water which now just covers the level of the burrows.

According to Arnold (*5*), the lower beds exposed in the quarries at Grand Ledge are at approximately the level of cycle "A" of Kelly (*6*), in the Saginaw group, Pottsville series. Although these burrows are of somewhat greater diameter and are considerably older than the lungfish burrows from Texas, they may have been made by the same genus, *Gnathorhiza*, which is reported from the Danville formation, lower Conemaugh (*7*), stratigraphically only slightly above the Michigan beds.

This discovery extends by almost a full period our knowledge of the aestivation of lungfish and reinforces Romer and Olson's suggestion of a very early separation of the aestivating line of lungfish from the more primitive, nonaestivating forms such as *Ceratodus* and *Epiceratodus*.

The presence of lungfish burrows raises again the question of why the Michigan coal basin, so rich in plant material, has so far revealed no terrestrial vertebrates and only a few scraps of aquatic vertebrates. Except for a pleuracanth spine (*8*) and several other undescribed fish spines, no other vertebrate remains have been reported from the Pennsylvanian of Michigan.

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References

1. A. S. Romer and E. C. Olson, *Breviora* **30**, 1 (1954).
2. W. Langston, Jr., *Bull. Nat. Museum Can.* **187**, 1 (1963).
3. P. P. Vaughn, *Los Angeles County Museum Contrib. Sci.* **80**, 1 (1964).
4. H. W. Smith, *Ecology* **12**, 164 (1931).
5. C. A. Arnold, *Contrib. Museum Paleontol. Univ. Michigan* **8**, 131 (1949).
6. W. A. Kelly, *Ann. Rept. Michigan Geol. Surv. Publ. 40, Geol. Ser. 34*, p. 149 (1936).
7. A. S. Romer and H. J. Smith, *J. Geol.* **42**, 700 (1934).
8. J. A. Dorr, *Papers Michigan Acad. Sci. Arts Letters* **42**, 99 (1957).

23 February 1965

Drosophila Phenol Oxidases

Abstract. *The phenol oxidase systems of* Drosophila melanogaster *arise from at least four protein components. One A component plus the P preparation yields tyrosinase, while the other two (A_2 and A_3) yield primarily 3,4-dihydroxyphenylalanine oxidases. Probably the four components represent specialized subunits of the oxidases produced.*

Beginning with a primary interest in the mechanisms for the regulation of the onset of melanin pigmentation about the end of the third day of the pupal life of *Drosophila melanogaster*, we have made an extensive study of the phenol oxidase system in this organism. The specific aspect of the problem that is considered here is derived from the original observation of Horowitz and Fling (*1*), who demonstrated that in adult flies tyrosinase activity in dilute extracts appears only after a period of incubation at 0°C. Evidence was presented for the existence of an inactive proenzyme and a separable activator protein. A similar situation was indicated for *Drosophila* larvae by Ohnishi (*2*) and for *Calliphora erythrocephala* diphenol oxidase by the extensive investigations of Karlson and collaborators (*3*). Thus, one phase of our program has been concerned with obtaining more specific information on the nature and number of components that enter into the formation of the phenol oxidases. The data presented here show that in *Drosophila melanogaster* there are at least four components. Three are designated A_1, A_2, and A_3; each gives rise to a phenol oxidase by reaction with the fourth component, designated P.

For preparation of A components a 1-g sample of wild-type (Oregon-R) pupae, aged 46 to 48 hours from puparium formation (*4*), was frozen at −80°C and then ground for 2 minutes at 0°C in a glass cone grinder with 20 ml of cold buffer [0.1*M* potassium phosphate, *p*H 6.3, containing 10 mg of disodium ethylenediaminetetraacetate (EDTA) per milliliter]. The homogenate was centrifuged at 0°C for 5 minutes at 18,000*g*, and the supernatant solution was subjected to fractionation with ammonium sulfate. The salt was added as a cold saturated solution in buffer, but the resulting *p*H 5.2 was not readjusted after saturation of the buffer with ammonium sulfate. Fractions retained after the addition of $(NH_4)_2SO_4$ were those resulting from saturations of 36 to 41 percent, 41 to 43 percent, 43 to 47 percent, and 47 to 56 percent, respectively. The first three fractions were washed once with ammonium sulfate solution, and each of the samples was dissolved in phosphate buffer (5 ml). Portions (0.05 ml) were subjected to electrophoresis on acrylamide gels for 3 hours at 4°C and 1.5 milliamperes per tube. The standard equipment and gel solutions obtained from Canalco (*5*) were used, with the exception that the lower gel was diluted to 6.5 percent.

For detection of A components on gels, a preparation of the fourth component, P, was obtained by homogenizing pupae in buffer as already described, but without addition of EDTA, which slows activation and alters the properties of P. The homogenate was centrifuged immediately, and the supernatant solution was adjusted quickly to 41 percent saturation with $(NH_4)_2SO_4$. The precipitate was washed with the salt at 41 percent saturation and then dissolved in phosphate buffer (10 ml per gram of pupae). Particulate matter was then removed by centrifugation at 105,000*g* for 1 hour at 0°C. The supernatant solution was used directly or stored

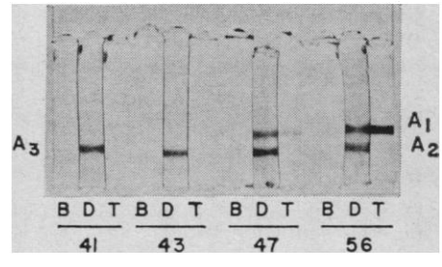

Fig. 1. Gel electrophoresis of A components from 48-hour pupae of *Drosophila melanogaster*. The origin of migration of proteins is at the bottom. Each gel of the four groups of three received 50 μl of solution from the $(NH_4)_2SO_4$ fraction indicated. Gels marked *B* were incubated with buffer and then with 3,4-dihydroxyphenylalanine. Those marked *D* and *T* were incubated first with the P fraction, then with the phenylalanine for *D* and with tyrosine for those marked *T*. Distinct A components are indicated by A_1, A_2, and A_3. Gels not containing A components but incubated with P and then the phenylalanine show no oxidase bands.